Promotion, Prediction and Prevention of Progression of Nephropathy in Type 1 Diabetes Mellitus

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The scope of the present review is to discuss the prognosis of diabetic renal disease, putative progression promoters and the possibilities for treatment and prediction of treatment efficacy. The recent changes in the incidence of diabetic nephropathy in Type 1 diabetes mellitus are discussed. Promoters of progression in diabetic nephropathy are evaluated, in particular arterial blood pressure, glycaemic control, albuminuria and cholesterol levels. Potential treatment modalities are discussed, with special focus on antihypertensive therapy, including a discussion of a specific renoprotective action of certain antihypertensive agents. Furthermore putative predictors of treatment efficacy are evaluated, demonstrating that the ability to lower the urinary albumin excretion rate after onset of treatment heralds a slow progression of the renal disease. The prognosis in diabetic renal disease has improved with an increase in median survival after onset of nephropathy from 6 to 15 years. This has exposed the importance of cardiovascular morbidity and mortality. The identification and treatment of cardiovascular risk factors has become essential. Although the prognosis has improved remarkably, the primary goal should be prevention of diabetic nephropathy, as it is unlikely that the increased risks associated with this complication can be eliminated. © 1998 John Wiley & Sons, Ltd.

Diabet. Med. **15: 900–919 (1998)**

KEY WORDS **Type 1 DM diabetic nephropathy; antihypertensive therapy; prognosis; survival**

Received 24 March 1998; revised 10 July 1998; accepted 10 July 1998

Introduction

Diabetic nephropathy is a serious long-term complication of Type 1 diabetes mellitus. After 25 years of diabetes, approximately 30–40 % of all Type 1 DM patients suffer from renal complications,[1] although the percentage has been declining since the 1930s.[2,3] Diabetic nephropathy is characterized by persistent albuminuria associated with a decline in glomerular filtration rate (GFR) and increasing arterial blood pressure.[4] Diabetes is the leading cause of end-stage renal disease in the United States.[5] The cost of caring for these patients alone currently exceeds US$ 1.8 billion per year in the US and is rising.[6] The presence of albuminuria heralds the presence of a more generalized vascular damage with microangiopathy in multiple organs especially glomeruli, retina and heart, and macroangiopathy. Among the major recent contributions to the clinical care of diabetic patients has been the recognition of the importance of hypertension for progression in diabetic nephropathy, and of the beneficial effect of antihypertensive treatment in postponing renal failure in Type 1 DM patients with diabetic nephropathy.[7,8]

The increased morbidity and mortality in Type 1 DM patients is mainly related to patients with diabetic nephropathy, as they suffer a dramatic increase in all cause and cardiovascular mortality.[1,9,10] The prognosis in patients with diabetic nephropathy has improved, but with great inter-individual variation, and no treatment has yet succeeded in arresting the deterioration in renal function.

The aims of this review have been to examine recent trends in the incidence of diabetic nephropathy in Type 1 DM patients, to identify promoters of progression in the disease, and especially progression promoters that could be subjected to therapeutic intervention, to evaluate the ability to prevent or diminish the progression, and to identify clinically useful short-term predictors of treatment effect on the long-term outcome. Finally the current prognosis in Type 1 DM has been assessed.

Methods

The references for this review were acquired via a MedLine and MedLars literature search, and from reference lists from the obtained papers. The search strategy was designed to obtain papers elucidating the specified aims, to discuss incidence, progression promoters and prognosis of diabetic nephropathy and treatment modalities: antihypertensive medication, lipid lowering agents, and dietary supplementation with fish oil, focusing on Type 1 DM. There has been no intention to exclude particular studies.

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 CCC 0742–3071/98/110900–20$17.50

Evaluation of Progression of Diabetic Nephropathy

The clinical diagnosis of diabetic nephropathy can be made in the presence of persistent albuminuria >300 mg $24\ h^{-1}$ or 200 μg min^{-1} in at least 2 of 3 sterile urine samples, in patients with a duration of diabetes ≥10 years, presence of diabetic retinopathy, and no clinical or laboratory evidence of kidney or urinary tract disease other than diabetic glomerulosclerosis.[8,11] When progression in diabetic nephropathy is evaluated the development of end-stage renal disease is the ultimate end point. As it takes many years to reach end-stage renal disease, clinical intervention trials in chronic progressive kidney diseases require other end points, like the rate of decline in GFR which has been approved as an end point by the Food and Drug Administration (USA) if measured over more than 2 to 3 years.[12] In order to obtain valid determination of the rate of decline in GFR the following requirements should be fulfilled in addition to sufficient duration of follow-up: the applied GFR method should have good accuracy and precision (as the ^{51}Cr-EDTA clearance), and repeated GFR measurements (approximately every 6 months) should be performed.[13] Substitutes for GFR measurements such as serum creatinine are not good enough for scientific purposes,[14] although estimates of GFR based on serum creatinine in large cohorts followed for many years may be useful.[15] In studies of shorter duration, changes in albuminuria can probably be regarded as a surrogate end point, associated with the long-term rate of decline in GFR (see below). Another possibility is to study changes in renal morphology especially in the early stages when GFR can be maintained by functional compensation (increase in intraglomerular pressure),[16] in spite of morphological progression of the disease. However this requires repeated biopsies, has been done in few studies,[17] and the primary morphologic end point needs to be determined.

Changed Incidence of Diabetic Nephropathy?

The discovery of insulin 75 years ago by Banting and co-workers (as recently described by Smidt-Nielsen[18] made it possible to manage the acute complications of Type 1 DM, but was followed by recognition of the late diabetic complications with angiopathy as a common denominator. Since then, substantial improvements in the management of diabetes have taken place. Several studies have shown a declining trend in the incidence of persistent proteinuria, comparing patients with an onset of diabetes in the 1930s to those with an onset in the 1960s.[1–3] Despite these improvements, the cumulative incidence of diabetic nephropathy in those with most recent onset (~1960) was still approximately 25 % after 25 years of diabetes and increasing with time. Then in 1994, a Swedish study by Bojestig *et al.*[19] reported a dramatic decline in the incidence of diabetic nephropathy in Type 1 DM patients diagnosed before the age of 15 years and between 1961 and 1980. Similar to previous studies, the patients with an onset of diabetes from 1961–65 had a cumulative incidence of diabetic nephropathy after 20 years with diabetes of 28 % but with an onset from 1971 to 1975 the 20 years incidence was only 5.8 % and none of the patients with onset from 1976 to 1980 had developed persistent proteinuria after 15 years.[19]

Was it also possible to demonstrate this positive trend at Steno Diabetes Center? We examined the 356 patients from the Hvidöre 1984 cohort[20] who had their diabetes diagnosed between 1965 and 1979 and resided of Copenhagen.[21] The patients were followed until 1991 and the incidence of diabetic nephropathy was studied. We were not able to corroborate the Swedish results. We observed a 15 years' cumulative incidence of diabetic nephropathy of 18 (SE: 4) %, 20 (4) % and 16 (5) %, in patients with an onset of diabetes from 1965 to 1969, 1970 to 1974, and 1975 to 1979, respectively (ns).[21] Furthermore 19–28 % of the patients had microalbuminuria in 1991 and were at increased risk for development of diabetic nephropathy. In contrast to the population-based study by Bojestig *et al.*,[19] our study was clinic based, which could lead to selection bias,[21] but although a population-based study is needed to determine the true incidence of diabetic nephropathy, a clinic-based study is well suited for the study of changes in incidence with time.

The patients in the Swedish study were older at onset of diabetes and the prevalence of men was higher, but this could not explain the large discrepancy between the studies. The two most striking differences between the two studies were the level of glycaemic control and the prevalence of smokers. Bojestig *et al.*[19] succeeded in obtaining a mean haemoglobin A_{1c} (HbA_{1c}) from 1980 to 1991 of ~7 % (normal range 3.2–6.0 %) similar to the level obtained in the intensively treated group in the Diabetes Control and Complications Trial (DCCT) study.[22] In comparison the mean HbA_{1c} was 8.8 % in our study (non-diabetic range 4.1–6.1) comparable to the conventionally treated group in the DCCT study.[22] Subsequently a comparison of HbA_{1c} methods including Steno Diabetes Center and Linköbing Hospital (Bojestig) revealed that the result in Linköbing was 1 % lower than the Steno result in the same samples.[23] This suggests that the differences between the mean level of HbA_{1c} in the Danish and Swedish studies was only 1. The successful improvement in glycaemic control was achieved by Bojestig *et al* by the formation of a special task force, providing education, psychosocial support, camps and education of the parents and included patients self-monitoring three times daily. An important, but not unique prerequisite for strict metabolic control is availability of test materials for blood glucose available free of charge in Sweden since the 1970s (the patients in Denmark have had a 50 % government subsidy since 1988). The importance of the special task force was stressed by the reporting of a cumulative incidence of persistent

microalbuminuria of 24.2 % after 15 years of diabetes (onset between 1976 and 1991) in a similar study by Rudberg *et al.*[24] from another paediatric department in Sweden in contrast to only 6–12 % microalbuminuria in the study by Bojestig *et al.*[19]

The DCCT[22] and several small studies summarized in the meta-analysis by Wang[25] have demonstrated the beneficial effect of intervention aiming at strict metabolic control as a means of preventing or delaying the development of diabetic nephropathy. However the DCCT study also demonstrated that it was extremely expensive to achieve and maintain the strict glycaemic control with a minimum of hypoglycaemic episodes during the 10-year study period. The need for the massive support was illustrated by a follow-up study, 1 year after termination of the original study, demonstrating that the HbA_{1c} level in the group previously receiving intensive therapy had increased to the level in the conventionally treated group (M. Steffes, personal communication).

In our study population the prevalence of smokers was 65 % compared to a decline to 20 % among diabetes patients in Sweden (H.J. Arnqvist personal communication). This could contribute to the declining incidence of diabetic nephropathy in the Swedish study as smoking has been associated with proteinuria in cross-sectional studies[26–29] and with the progression from normo- to microalbuminuria in prospective studies.[30,31]

The results obtained by Bojestig *et al.* may be unique. The progressive increase in the number of diabetic patients receiving treatment for end-stage renal disease in USA[32] and Europe[33] indicates that the marked decline in the incidence of diabetic nephropathy found by Bojestig *et al.*[19] is not a general finding. There is however a delay in time of approximately 10 years from the development of persistent proteinuria to the development of end-stage renal disease.[2] Furthermore the rise in patients receiving renal replacement therapy due to diabetes is partly due to an increase in the number of Type 2 DM patients developing end-stage renal disease and partly due to a change in referral of Type 2 DM patients with renal failure.

Several long-term studies have now demonstrated that the progression from microalbuminuria to diabetic nephropathy is delayed in normotensive microalbuminuric patients treated with angiotensin converting enzyme (ACE) inhibitors.[34–36] But as it was not a general policy to prescribe ACE inhibitors for normotensive microalbuminuric patients before 1991, neither in Sweden nor at Steno Diabetes Center, the use of ACE inhibitors cannot explain the declining incidence in Sweden.

In conclusion the incidence of diabetic nephropathy has declined since the 1930s but still 25 % of patients develop this complication after 25 years of diabetes. Recent intervention studies demonstrate that it is possible to reduce the incidence of diabetic nephropathy if very strict metabolic control is achieved and if ACE inhibitors are used in microalbuminuric patients. These interventions will be expensive, but the costs should be compared with the savings by reducing the number of patients developing end-stage renal disease[37,38] and the gain in quality of life. Future research will have to evaluate whether these interventions will work as satisfactorily in the routine clinic as in clinical trials and whether the prerequisites for the cost effectiveness are fulfilled.[37,38]

After 25 years with diabetes the incidence of nephropathy is about 25 %. This can be improved if:

- very strict glycemic control is achieved;
- ACE inhibitors are used in persistently microalbuminuric patients;
- other risk factors such as smoking are taken into consideration.

Progression promoters in Diabetic Nephropathy

Diabetic nephropathy is characterized by relentless decline in GFR, and an increasing blood pressure and proteinuria. At onset, GFR is high or normal; preceding microalbuminuria glomerular hyperfiltration is often present. The GFR starts to decline when albuminuria exceeds approximately 300 mg $24\ h^{-1}$, although the variation in rate of decline between individuals is large, 0–20 ml min^{-1} yr^{-1},[4,39,40] with time to death ranging from 2 to 32 years.[1] Understanding the factors responsible for this variation in progression, i.e. identification of progression promoters, would help direct future therapies.

Whether arterial blood pressure rises before the GFR starts to decline,[41] suggesting a causative role of blood pressure for the development of diabetic nephropathy, or whether the renal disease causes blood pressure to rise,[30,42] is a matter of debate. It is clear that hypertension, or a rise in blood pressure within the normal range compared to previous levels, is common in the early stages of diabetic nephropathy.[43] The hypertension is related to sodium retention and volume expansion. Normal values of serum renin suggests that serum renin is inappropriately suppressed.[44,45] The changes are accompanied by characteristic morphological changes in the kidney: hypertrophy of both glomerular and tubular elements; thickening of glomerular and tubular basement membranes; accumulation of mesangial matrix (most characteristically the nodular glomerulosclerosis described by Kimmelstiel and Wilson in 1936); hyalinosis of efferent and afferent glomerular arterioles and later glomerular occlusion and tubulointerstitial fibrosis. Structural and functional changes accompany each other.[46] The precise structural changes underlying the leakiness of the filtration barrier have not been identified.[46] Albuminuria is associated with widening of the glomerular basement membrane and of the glomerular epithelial foot processes but also with changes in the quality of the former: loss of charge and, later, size selectivity.[47–49] GFR is determined by the ultrafiltration pressure gradient (hydraulic and oncotic pressure) across the filtration barrier, and the ultrafiltration coefficient K_f (the product

of the filtration surface area and the hydraulic permeability of the glomerular capillary of the filtration surface area and the hydraulic permeability of the glomerular capillary wall). Reduction in GFR correlates with decreased filtration surface area, due to occluded glomeruli and mesangial expansion exceeding glomerular hypertrophy, compromising the structure of the capillaries. In the early stages of diabetic nephropathy a loss of ultrafiltration capacity can be offset by an increase in glomerular capillary hydraulic pressure.[50]

Which factors promote progression in diabetic nephropathy? We addressed this question in two studies. The first[51] focused on the natural history of diabetic nephropathy, i.e. the course of diabetic nephropathy without any treatment except for insulin. The second[52] described progression promoters during 10 years' treatment with an ACE inhibitor. In both studies the impact of arterial blood pressure and albuminuria were evaluated and in the second study we also evaluated the effect of glycaemic control, and serum cholesterol. The studies demonstrated the need for repeated measurements of GFR when monitoring patients with diabetic nephropathy in order to identify high risk patients with a fast decline in GFR.

Natural History

Previous studies demonstrated a mean rate of decline in GFR of 10–15 ml min^{-1} yr^{-1}, ranging from 0 to 25.[4,39,40] It was shown that Type 1 DM patients with heavy proteinuria (>3g 24 h^{-1}) and marked renal structural lesions have the worst prognosis,[53] as all such patients died within 11 years. The results of previous studies of the impact of systemic blood pressure on progression in diabetic nephropathy have been conflicting.[4,39,40] In a study of early diabetic nephropathy in Type 1 DM we followed 41 patients with serum creatinine <150 μmol l^{-1} and arterial blood pressure at baseline below 160/100 mmHg for 36 (15–66) months with two GFR determinations per year. Albuminuria was determined during the clearance period and arterial blood pressure was measured during each investigation and at visits to the outpatient clinic every 3–4 months. The rate of decline in GFR ranged from −2.1 to 17 ml min^{-1} yr^{-1} (mean 7.1) and was correlated with diastolic blood pressure ($r = 0.52$, $p < 0.001$) and albuminuria ($r = 0.34$, $p < 0.05$). In a multivariate regression analysis only diastolic blood pressure was correlated with decline in GFR when all patients were included; albuminuria was correlated with the decline in GFR only when patients with a diastolic blood pressure below the mean value (89 mmHg) were included ($r = 0.51$, $p < 0.01$). This suggests that systemic blood pressure and albuminuria accelerate the progression of diabetic nephropathy. Albuminuria seems only to be of significance in patients with normotension (see Figure 1). Age at onset of diabetes, duration of diabetes and insulin dose were not related to the fall in GFR. The correlation coefficients indicated

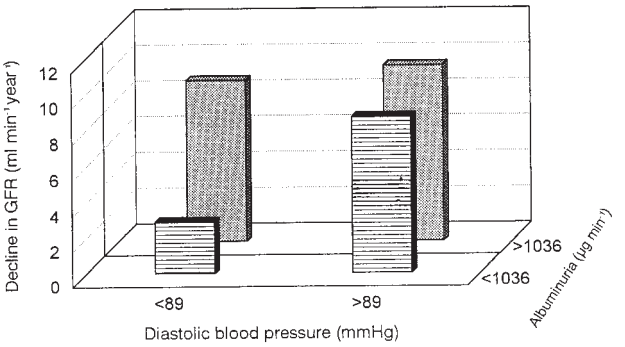

Figure 1. Impact of diastolic blood pressure and albuminuria on progression of diabetic nephropathy in 41 Type 1 DM patients (adapted from Rossing *et al.*[51])

that the variability in rate of decline in GFR is only partly explained by these factors but we were not able to evaluate the impact of other putative progression promoters, especially glycaemic control and serum cholesterol.

Progression Promoters During Antihypertensive Treatment

Several studies have demonstrated the beneficial effect of antihypertensive treatment on decline in GFR; a wide range in rate of decline in GFR is observed even during antihypertensive treatment. We evaluated progression promoters during 10 years' treatment with an ACE inhibitor in a prospective study in hypertensive Type 1 DM patients with nephropathy.[52] Eighteen patients were followed for at least 10 years from onset of nephropathy or until death. Nearly all patients received a diuretic in addition to the ACE inhibitor captopril, in order to control hypertension and/or treat oedema. The rate of decline in GFR ranged from −1.6 to 9.1 ml min^{-1} yr^{-1} (mean 4.4 ml min^{-1} yr^{-1}), and correlated with mean arterial blood pressure ($r = 0.58$, $p < 0.01$), albuminuria ($r = 0.67$, $p < 0.005$), HbA_{1c} ($r = 0.69$, $p < 0.005$) and serum total cholesterol ($r = 0.51$, $p < 0.05$) in univariate analysis. A stepwise multiple regression analysis revealed that only albuminuria and HbA_{1c} during antihypertensive treatment correlated independently with the rate of decline in GFR, explaining two-thirds of the deterioration in kidney function.

Arterial Blood Pressure

The association between arterial blood pressure and decline in GFR was originally described by Mogensen,[39] who found that diastolic blood pressure at the end of follow-up was correlated with decline in GFR prior to the onset of antihypertensive treatment. In two other studies this was not confirmed,[4,40] but numbers were small in all three. Systemic and intraglomerular hypertension enhances the development of diabetic glomerulopathy and accelerates the decline in GFR during antihypertensive treatment[54–56] as in our study. The lack of an independent

correlation with blood pressure in our treatment study is probably due to the well-controlled blood pressure, absence of severe hypertension and a narrow range of blood pressure, as also found by Nyberg.[57] Experimental studies by Brenner *et al*.[58,59] show that systemic hypertension is transmitted to the single glomerulus leading to glomerular hypertension and hyperperfusion. Intraglomerular hypertension has also been shown in normotensive streptozotocin diabetic rats.[60] Other studies have also demonstrated that haemodynamic factors accelerate the development of glomerulopathy in diabetic animals.[61,62] Conversely antihypertensive therapy reduces albuminuria and reduces the rate of decline in GFR.[7,8,63,64] In Type 1 DM patients with diabetic nephropathy, impaired autoregulatory capacity contributes to the transmission of systemic hypertension to the glomerulus.[65] Recently Cortes *et al*.[66] have shown in experimental studies, that oscillations in pressure, as seen in the glomerulus in the absence of autoregulatory pressure protection, can induce production of extracellular matrix in mesangial cells, probably mediated through stretch induced stimulation of TGF-β1 production, thus linking haemodynamic alterations with structural modifications.

Proteinuria

Proteinuria is usually considered a marker of the extent of glomerular damage, but recent studies in various experimental animal models suggest that proteinuria *per se* may contribute to glomerular damage.[67] It remains to be established which of the filtered proteins, albumin, lipoproteins, etc., play the major role. In many of these models, proteinuria seems to precede structural changes. It is suggested that the mesangial cells may proliferate and synthesize mesangial matrix when they are exposed to an abnormal plasma protein traffic due to leakiness of the glomerular filtration barrier. Among other proteins, lipoproteins, especially oxidized LDL, stimulate production of mesangial matrix[68] and circulating LDL may increase filtration barrier permeability through binding with glycosaminoglycans in the glomerular basement membrane.[69] Furthermore the increased amount of protein in the ultrafiltrate may exceed the tubular epithelial cell reabsorptive capacity, leading to formation of casts and subsequent inflammatory reactions. With declining renal function, the proteinuria changes composition, due to loss of selectivity of the glomerular filtration barrier. An increasing ratio of IgG/albumin clearance thus reflects loss of size selectivity. We have observed an association between albuminuria and loss of kidney function, confirming the original observations by Watkins *et al*.[53] demonstrating the worst prognosis in patients with severe proteinuria (>3 g 24 h^{-1}). This was further supported by Williams *et al*.,[70] in patients with mild to moderate renal failure of different aetiologies including diabetic nephropathy and by the Modification of Diet in Renal Disease study in 850 patients with chronic non-diabetic renal diseases.[71] Furthermore, intervention that has ameliorated the progression of diabetic renal disease has always been associated with a reduction in proteinuria.[7,63,64,72,73] Although our findings are in accordance with the concept of glomerulosclerosis as a consequence of the increased plasma protein traffic through the mesangium, reflected by proteinuria, they could likewise be explained by increased selectiveness of the filtration barrier or lowering of filtration pressure leading to reduced proteinuria. The covariance of arterial blood pressure and proteinuria has to be recognized,[74,75] and makes it difficult, based on correlations, to establish whether hypertension and/or albuminuria is the primary progression promoter.

Glycaemic Control

In general there is agreement on the beneficial effect of strict glycaemic control in delaying development of diabetic kidney disease in normoalbuminuric patients.[22,25] Conversely it is believed that once proteinuria has become persistent, glycaemic control has no impact on the deterioration in renal function.[2,4,40,63,76,77] However, this assumption is based on studies using inappropriate methods for monitoring kidney function (serum creatinine) and long-term glycaemic control (blood glucose), and often very few patients are studied. Originally Nyberg *et al*.[57] demonstrated a correlation between the rate of decline in GFR and haemoglobin A_{1c} in 18 hypertensive Type 1 DM patients suffering from diabetic nephropathy treated aggressively with antihypertensive medication followed for 18 months. Our long-term study[52] confirmed and extended this observation by demonstrating that variation in glycaemic control and albuminuria could explain two-thirds of the variation in the rate of decline in GFR (see Figure 2). Recently Mulec *et al.* have also found an association between glycaemic control and progression in diabetic nephropathy.[78] It is not known whether improvement in glycaemic control has any beneficial effect on the decline in renal function. The only long-term study conducted so far has been negative, but included only 6 patients.[79] This question needs to be addressed in future studies, although it is a general

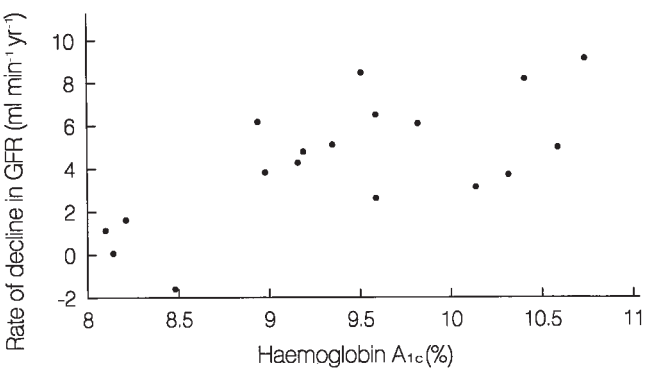

Figure 2. Haemoglobin A_{1c} and rate of decline in GFR during long-term ACE inhibitor treatment in hypertensive Type 1 DM patient with diabetic nephropathy ($n = 18$; $r = 0.69$, $p < 0.01$) (adapted from Parving *et al*.[52])

experience that it is difficult to near-normalize blood glucose in Type 1 DM patients with nephropathy. The impact of high blood glucose may be due to a direct effect of glucose on mesangial cells leading to an increased production of mesangial matrix proteins, mediated by transforming growth factor-β1 (TGF-β1) or other growth factors, hormones or cytokines.[80] Furthermore high blood glucose levels lead to binding of glucose to structural proteins, i.e. the formation of advanced glycosylation end products. These modified proteins are capable of forming covalent bonds with other proteins followed by changes in cross-linking of proteins and increased leakiness of the glomerular basement membrane.[81]

Hyperlipidaemia

It has been suggested that hyperlipidaemia promotes progression in chronic renal diseases once an initial event has damaged the glomerular filtration barrier, allowing the passage of lipids and lipoproteins into the mesangium.[69] The process has been compared to atherosclerosis with the formation of foam cells from macrophages as an important step. It has also been suggested that hypercholesterolaemia, through an effect of the renal eicosanoids, can induce intraglomerular hypertension. The potential influence of oxidized lipoproteins has been discussed above. We observed a correlation between serum cholesterol and rate of decline in GFR, although the association disappeared when corrected for other progression promoters. Our data confirm the study by Mulec *et al.*[82] of 31 Type DM patients with diabetic nephropathy followed for a mean of 18 months. Correction for other progression promoters was performed in a later publication[83] showing serum cholesterol to be an independent progression promoter. In a study of 436 patients with intermittent or persistent proteinuria, Krolewski *et al.* demonstrated baseline serum cholesterol and systemic blood pressure to be associated with loss of renal function (serum creatinine) over 3 years.[84] We also demonstrated a significant, although weak, correlation between serum cholesterol and decline in GFR in 29 normotensive Type 1 DM patients with nephropathy followed for 12 months.[85]

Protein Intake

The patients in our studies received a recommended 'diabetic' diet comprising 15–20 % of protein. Protein intake was not restricted, and the patients from the ACE inhibitor study[52] had a mean protein intake of 1.1 g kg^{-1} day^{-1} during the first 3 years. This was not correlated to the rate of decline in GFR. In contrast, experimental studies have demonstrated protein intake to be related to the progression in glomerulopathy due to a lowering of intraglomerular pressure through constriction of the afferent arteriole (see below).

Several studies have related smoking to the initiation and progression of diabetic nephropathy. The mechanism for this association is not clear. It could be related to induction of changes or oscillations in systemic/glomerular blood pressure, toxic substances in the tobacco, or interactions with the autonomic nervous system.

In conclusion, the natural course of decline in kidney function during diabetic nephropathy is highly variable. Antihypertensive therapy can slow the average rate of progression, but the inter-individual variation is still large. This variability can at least partly be explained by promoters of progression.

Progression promoters in diabetic nephropathy may be listed as follows:

- arterial hypertension
- albuminuria
- glycaemic control
- dyslipidaemia
- dietary protein intake
- smoking
- ACE insertion detetion polymorphism.

Predictors of Efficacy of Antihypertensive Treatment on Progression

It takes several years to determine the rate of decline in GFR with certainty, that is, the effect of any intervention on the primary end point: loss of kidney function. From a clinical point of view this is unsatisfactory. The identification of early predictors of long-term efficacy after onset of a new treatment modality would be important, as this could allow the early identification of patients in need of an intensified or changed therapeutic regimen. In clinical trials the identification of such predictors (surrogate end points) would allow faster evaluation of new drugs, although it must be recognized that a predictor of the efficacy of one type of therapy might not be useful in the evaluation of another type of therapy.

Is it possible to identify a predictor for the efficacy of antihypertensive therapy? To answer this question we analysed the data from two prospective studies in Type 1 DM patients with diabetic nephropathy. Our aim was to generate a hypothesis based on the first study and test it in the second study. The primary study[86] included 20 patients followed for at least 18 months prior to onset of conventional antihypertensive treatment (diuretics and cardioselective β-blockers, hydralazine was added if necessary, and 1 patient received an α-blocker). The patients were followed for 3 years on treatment. The rate of decline in GFR, measured semi-annually, was used as the primary end point. As putative predictors we evaluated arterial blood pressure, GFR, albuminuria and adjusted albuminuria (albuminuria/GFR). These variables were calculated for the last year before antihypertensive treatment and for the first year during treatment. The change in these variables was also evaluated comparing values from the first year during treatment with values from

the last year prior to onset of treatment. Antihypertensive treatment reduced all variables significantly, and the long-term rate of decline in GFR ranged from −1 to 11 ml min^{-1} yr^{-1} (mean 3.6). The analysis demonstrated that only the initial change in adjusted albuminuria was associated with the long-term rate of decline in GFR (r = 0.46, $p < 0.05$). This indicates that a reduction in albuminuria with preservation of GFR is a predictor of a desirable outcome. Furthermore, reduction in relative change in adjusted albuminuria was also associated with a reduction in rate of decline in GFR when the decline in the treatment period was compared to the pre-treatment period. This leads us to hypothesize that an initial reduction in adjusted albuminuria after onset of conventional antihypertensive treatment is predictive of long-term preservation of renal function.

This hypothesis was tested in the second study including 18 patients followed for 3 years from the onset of antihypertensive treatment with the ACE inhibitor captopril.[87] In addition to the above mentioned putative predictors it was possible in this study that we evaluated the influence of other putative progression promoters: HbA_{1c}, serum cholesterol concentration, protein intake, and urinary sodium excretion. The study demonstrated that low levels of albuminuria shortly after onset of antihypertensive treatment were associated with an attenuated rate of decline in GFR. Stronger correlations were obtained if the relative change in albuminuria or adjusted albuminuria (albuminuria/GFR) were used (see Figure 3). There were no correlations between rate of decline in GFR and serum concentrations of HbA_{1c}, cholesterol, protein intake, and sodium excretion, and these variables remained unchanged during the study period. Thus in the two independent studies we found that initial reduction in urinary albumin excretion or adjusted albuminuria could serve as a clinical guideline predicting a beneficial effect of antihypertensive treatment on the long-term decline in renal function.

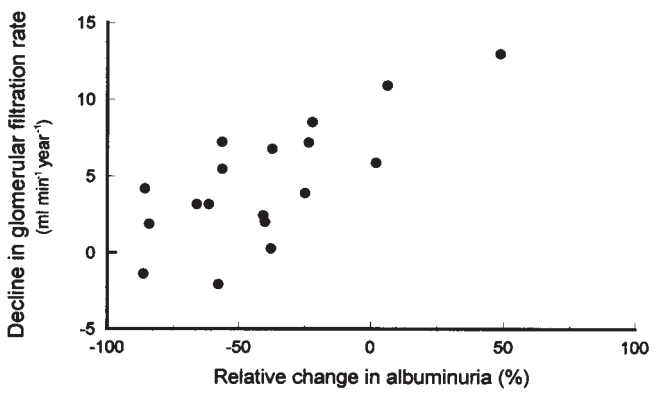

Figure 3. Correlation between relative change in albuminuria and decline in glomerular filtration rate (r = 0.73, $p < 0.001$). Albuminuria was measured at baseline and during the first year after start of antihypertensive treatment, respectively. Glomerular filtration rate was measured during 3 years of antihypertensive treatment in 18 Type 1 DM patients with diabetic nephropathy (adapted from Rossing *et al.*[87])

Our findings are supported in the literature. Apperloo *et al.*[88] demonstrated that the reduction in proteinuria after 12 weeks' therapy correlated with the long-term rate of decline in GFR in 29 proteinuric non-diabetic patients treated for 2 years with atenolol or enalapril. The number of patients in all these studies was rather small which may have contributed to the lack of correlation with arterial blood pressure or GFR. However similar findings were reported by the Collaborative Study Group of Angiotensin Converting Enzyme Inhibition with Captopril in Diabetic Nephropathy in a study of 409 Type 1 DM patients with diabetic nephropathy.[89] The Modification of Diet in Renal Disease study (MDRD study) of 840 patients with non-diabetic renal diseases likewise demonstrated the initial decline in proteinuria to be associated with the subsequent rate of decline in GFR.[71] Furthermore the MDRD study demonstrated a correlation between rate of decline in GFR and proteinuria, arterial blood pressure, black race, serum HDL cholesterol, diagnosis of polycystic kidney disease, and serum transferrin level at baseline.[71] In a 5-year study of Type 2 DM patients with microalbuminuria randomized to treatment with enalapril or placebo the decline in renal function (evaluated by serum creatinine) was related to albuminuria, arterial blood pressure, and total cholesterol at baseline.[90]

Treatment which successfully reduces progression in diabetic kidney disease both experimentally[55,58,59] and in clinical trials[7,63,64,72,73] has been associated with a reduction in proteinuria during the intervention period; conversely, treatment, e.g. strict metabolic control, lacking an antiproteinuric effect had no beneficial impact on the rate of progression of renal failure in Type 1 DM patients with diabetic nephropathy.[79]

The reduction in albuminuria after start of ACE inhibition or conventional antihypertensive treatment frequently combined with diuretics must reflect functional or structural factors contributing directly or indirectly to the deterioration in renal function. The rapid onset of the effect suggests functional factor. However the effect may increase with time, suggesting a structural component too.[91] The antiproteinuric effect may be due to either a reduction in glomerular capillary hydraulic pressure, or enhanced selectivity of the glomerular capillary barrier, or both. Increased glomerular capillary hydraulic pressure has been shown to accelerate the development of diabetic glomerulopathy[58] and antihypertensive medication reduces the intraglomerular pressure, albuminuria and the severity of experimental diabetic glomerular lesions.[55] In some experimental studies the antiproteinuric effect is solely present if the reduction in systemic blood pressure leads to a decrease in intraglomerular hydraulic pressure[55,92] although other studies could not confirm this.[93] Inhibition of angiotensin converting enzyme prevents the formation, and thus the action, of angiotensin II on the glomerular arterioles, particularly the efferent. This leads to a dilation of the efferent arteriole thereby diminishing

the intraglomerular pressure irrespective of the effect on systemic hypertension.

The size and charge selectivity of the glomerular capillary filtration barrier is impaired in patients with diabetic nephropathy compared to diabetic patients with normo- and microalbuminuria.[47,49,94] Antihypertensive treatment with an ACE inhibitor has been shown to improve both size and charge selective properties.[95,96] Whether this effect is specific for ACE inhibitors is not known. Reddi *et al.*[96] demonstrated that the antiproteinuric effect of ACE inhibition was associated with preservation of heparan sulfate in the glomerular basement membrane in rats. Heparan sulphate is a glycosaminoglycan and a major contributor to the negative charge of the glomerular basement membrane, and thus of the charge selective properties of the glomerular capillary barrier. In a study by Morelli *et al.*[95] in Type 1 DM patients with diabetic nephropathy, enalapril could ameliorate the size selective defect in the glomerular capillary barrier by reducing the average radius of small restrictive pores, as well as of the large non-restrictive pores.

The salient point is whether proteinuria can be used as a predictor of the efficacy of any kind of treatment in renal diseases. This is not yet clear. Almost all studies reporting albuminuria to be a predictor of deterioration in renal function have used ACE inhibitors or conventional antihypertensive agents. It is possible that some agents may protect glomeruli from the glomerulosclerotic process without affecting the proteinuria. Conversely Tanaka *et al.*[97] demonstrated in an animal model that the proteinuria could be reduced without preventing the glomerulosclerotic process. The general effectiveness would depend on whether proteinuria *per se* is just a marker or a progression promoter.

Repeated renal biopsies and morphometric and biochemical techniques may be needed to identify early changes in morphology or gene expression after onset of a new treatment, which can be correlated with long-term outcome in renal function.[16,98] However such predictors of efficacy requiring serial biopsies would only apply to intervention trials and not to the routine clinic.

Genetics

The findings of familial clustering of diabetic nephropathy,[99,100] and the fact that only a subgroup of patients seems to be susceptible to it, suggests that hereditary causes may be involved in its pathogenesis. Genetic markers of susceptibility to diabetic nephropathy have been sought. Several studies have investigated the role of an insertion(I)/deletion (D) polymorphism in the gene encoding for the angiotensin converting enzyme, which is associated with the plasma level of ACE and with an increased incidence of myocardial infarction in the non-diabetic population.[101] Although initial case-control studies indicated that the II-genotype was a marker for reduced risk of development of diabetic nephropathy, subsequent results have been conflicting.[102,103] A recent metanalysis suggested that the II-genotype tended to be protective in IDDM patients (odds ratio 0.72 (95 % CI: 0.51 to 1.01)) but was protective in Japanese Type 2 DM patients and of no importance in Caucasian Type 2 DM patients.[104] Whereas this polymorphism probably does not play a major role in the initiation, it may relate to the progression of diabetic[105] and non-diabetic renal[106,107] disease during antihypertensive treatment. We found that the DD genotype independently influenced the sustained rate of decline in GFR, i.e. acted as a progression promoter in IDDM patients during ACE inhibitor treatment.[105] Others have confirmed that the DD genotype seems to be a progression promoter in Type 1 DM,[108,109] and Type 2 DM.[110] This suggests that it will be possible to predict the beneficial effect of a therapeutic intervention from knowledge about the genetic constitution, and perhaps even to qualify selection among therapeutic interventions based on such knowledge.

In summary our findings suggest that an initial reduction in albuminuria or low levels of albuminuria after the onset of antihypertensive medication (surrogate end points) predicts long-term preservation of kidney function (principal end point). This has been confirmed in studies of diabetic and non-diabetic renal diseases. Whether the predictive effect can be seen with other treatment modalities needs urgently to be determined. Future research should also clarify if a sudden increase in albuminuria conversely could be used as a predictor of an ominous outcome. It is also likely that future predictors of treatment efficacy will include genetic markers playing a role in initiation and/or progression of diabetic nephropathy.

- A reduction in albuminuria after onset of antihypertensive treatment predicts a beneficial long-term outcome on renal function.
- Future predictors of a beneficial outcome may include genetic markers.

Prevention of Progression

Untreated diabetic nephropathy is lethal. However, the therapeutic goal in patients with diabetic nephropathy is not only to prevent the progression to end-stage renal disease but should encompass the frequent coexistence of generalized microangiopathy, involving other organs such as the eyes and the heart in order to successfully improve the prognosis. Unfortunately no therapeutic regimen has been able to eliminate the deterioration in renal function and the increased risk for early death despite major improvements within this field. Thus the primary goal remains the prevention of the development of diabetic nephropathy. In the following sections intervention with antihypertensive agents, lipid lowering agents, diet (fish oil), improved metabolic control, and dietary protein restriction will be discussed.

Antihypertensive Therapy

The Importance of Antihypertensive Treatment

Although the association between hypertension and diabetic nephropathy was described by Kimmelstiel and Wilson in 1936, the adverse effect of hypertension in diabetic (and non-diabetic) renal disease was not appreciated until the late 1970s.[111] A high blood pressure was considered advantageous in the 1950s and 1960s![111] Antihypertensive treatment has become recognized as the most successful treatment modality in diabetic nephropathy only after the initial studies of antihypertensive medication by Mogensen[7] and Parving,[8] where albuminuria decreased and the rate of decline in GFR was reduced from >10 to <5 ml min^{-1} $year^{-1}$. These studies used conventional antihypertensive agents (mostly β-blockers in combination with diuretics). It was estimated that the time to development of end-stage renal disease was extended from 7 to 21 years[63] and it was demonstrated that the beneficial effect on the rate of decline in GFR was maintained for at least 10 years.[112] Other studies have confirmed these results.[57,113]

The initial studies were self-controlled, the rates of decline in GFR before and after antihypertensive treatment were compared. Due to the remarkable effect on deterioration in kidney function it has since been considered unethical to perform a randomized controlled trial in hypertensive patients with diabetic nephropathy comparing antihypertensive medication with placebo. The success of antihypertensive medication in reducing rate of decline in GFR makes it more difficult to study other intervention modalities as the potential gain has been reduced.

When to Treat?

It has been intensively discussed at what blood pressure level the antihypertensive treatment should be commenced and what should be the goal for the treatment. This question has not been addressed specifically, but the attitude has been increasingly aggressive. Recently it was recommended that intervention, initially non-pharmacological, should start when blood pressure is >140/90 mmHg. In diabetic patients with end organ damage such as nephropathy the goal should be <130/85 mmHg, and if that is well tolerated a further careful lowering of blood pressure to 120/80 mmHg is suggested.[114,115] This would include >80 % of patients with diabetic nephropathy.[116] In the recently published guidelines on management of diabetes in Denmark it was recommended to keep blood pressure <140/85 mmHg in all diabetic patients <60 years of age.[117] The equivocal issue is whether to treat normotensive patients with diabetic nephropathy. Recent studies have not been able to document that antihypertensive effect is beneficial regarding hard end points in normotensive patients with diabetic nephropathy.[118,119] Eight years of treatment with an ACE inhibitor could not reduce the rate of decline in GFR compared to placebo in normotensive patients, although albuminuria was less in the ACE inhibitor group.[119] Recent guidelines[120] recommend ACE inhibition in normotensive patients with microalbuminuria as this reduces the risk for progression to persistent albuminuria.[34,35,121] Since the treatment will continue if diabetic nephropathy develops, all patients will eventually be treated with an ACE inhibitor.

The Effect of Various Antihypertensive Agents

Metanalyses by Weidmann *et al.*[122] and Kasiske *et al.*[123] including short- and long-term studies of Type 1 DM and Type 2 DM patients with micro- and macroalbuminuria demonstrated an enhanced antiproteinuric effect of ACE inhibitors over β-blockers and diuretics. This effect appears to be independent of a reduction in systemic blood pressure. With increasing reduction in arterial blood pressure the difference between the agents was reduced.[122] The calcium antagonists should be split into capsular nifedipine (short acting dihydropyridine) where a 21 % increase in albuminuria/proteinuria was observed whereas other calcium antagonists reduced albuminuria/proteinuria by 24 %.[122] In a recent metanalysis of diabetic and non-diabetic patients by Maki *et al.*[124] the effect on GFR was the same for different classes of antihypertensive agents, except for an increased loss in GFR with dihydropyridine calcium antagonists (such as nifedipine). The latter analysis only included studies of more than 6 months' duration; no studies in Type 1 DM patients with diabetic nephropathy on calcium antagonists were included.

What is the long-term effect of ACE inhibitors in hypertensive Type 1 DM patients with diabetic nephropathy? We evaluated this in an uncontrolled prospective study of 18 patients followed for a mean of 9 years on treatment.[52] For the entire observation period a reduction in arterial blood pressure and albuminuria was obtained together with a low rate of decline in GFR of 4.4 ml min^{-1} $year^{-1}$ (SE 0.7) (see Figure 4). There was an initial drop in GFR (6.1 ml min^{-1} 1.73 m^{-2} in 3 months). This is at least partly reversible[125] and thus probably haemodynamically mediated, related to the impaired autoregulation in the kidney.[65] Only two patients developed end-stage renal disease after 10 year's nephropathy. In an analysis made after 6 years of treatment, conventional antihypertensive therapy in a matched but historical group of patients proved as efficacious as ACE inhibition in keeping a low rate of deterioration. The rate of deterioration in GFR was 4.5 (SE 0.6) ml min^{-1} y^{-1} in the ACE inhibitor group vs 4.1 (0.7) in the conventional group.[126]

Two studies[72,118] have suggested that ACE inhibitors specifically offer renal protection, above and beyond what could be expected from the blood pressure reduction alone, at least in patients with advanced diabetic nephro-

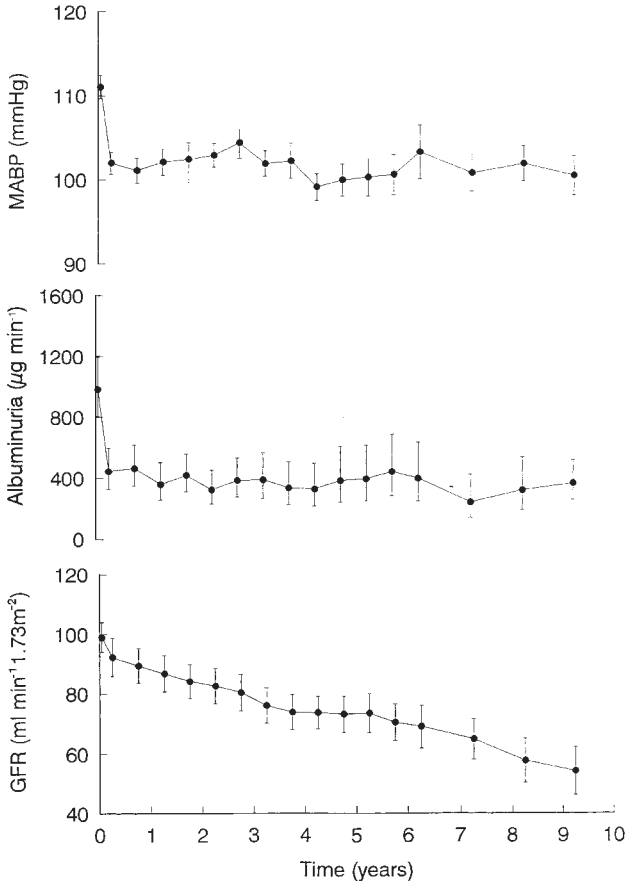

Figure 4. Mean course of mean arterial blood pressure, albuminuria, and GFR during long-term ACE inhibitor treatment in hypertensive Type 1 DM patients with diabetic nephropathy. Bars represent SEM (×/÷ antilog SE for albuminuria) (adapted from Parving *et al.*[52])

pathy. Originally Björck *et al.*[72] reported that treatment with an ACE inhibitor protected renal function better than treatment with a β-blocker in moderately advanced diabetic nephropathy (mean GFR 47 ml min$^-$ 1.73 m^{-2}). They observed a decline in GFR of only 2 ml min^{-1} year^{-1} in the ACE inhibitor group (the lowest reported rate of decline in GFR on ACE inhibition). This was supported by The Collaborative Study Group of Angiotensin Converting Enzyme Inhibition with Captopril in Diabetic Nephropathy[118] demonstrating, in a double-blind randomized study of 409 patients, a significant risk reduction (68 %, 95 % Cl 39 to 83 %) in time for occurrence of doubling of serum creatinine in 102 patients with baseline serum creatinine >133 μmol^{-1}, whereas the risk reduction was non-significant in 307 patients with baseline serum creatinine <133 μmol^{-1}. It is possible that an increase in intraglomerular pressure, in order to compensate for the reduction in the filtration coefficient K_f due to glomerular closure with declining renal function, makes ACE inhibition particularly beneficial in advanced diabetic nephropathy. It is equally possible that the lack of effect in the patients with normal serum creatinine was due to the selected end point – time to doubling of creatinine – which is unlikely to occur within the observation period in patients with normal kidney function.[118] Our observation of a low rate of decline in GFR of 4.4 m min^{-1} year^{-1} during ACE inhibition is in accordance with most studies of more than 18 months duration using either ACE inhibition or conventional antihypertensive treatment.[7,57,63,127,128]

Are calcium channel blockers as good as ACE inhibitors in preserving kidney function in patients with diabetic nephropathy? In general the studies with calcium antagonists in diabetes have suffered from being either short, uncontrolled, mixing Type 2 DM and Type 1 DM, including normo-, micro- and macroalbuminuric patients, or including few patients.[129–136] We have initiated a 4-year randomized study in 49 hypertensive Type 1 DM patients with diabetic nephropathy comparing the ACE inhibitor lisinopril with the calcium antagonist nisoldipine. We have recently reported the results of the first 12 months, focusing on the effect on albuminuria.[137] In the ACE inhibitor group the albuminuria decreased from 1.5 to 0.8 g 24 h^{-1}, whereas no significant change was observed in the calcium antagonist group (from 1.0 to 1.2 g 24 h^{-1}) ($p < 0.05$ comparing changes in the two groups). Fractional clearances of albumin changed similarly. In contrast to the potentially beneficial effect of a reduction in albuminuria, a larger decline in GFR after 1 year was seen in the ACE inhibitor group: from 85 to 73 ml min^{-1} 1.73 m^{-2} compared to the calcium antagonist group: from 84 to 80 ($p < 0.05$ comparing changes in the two groups) (see Figure 5). We observed a fast initial decline in GFR during the first 6 months compared to the following 6 months in the ACE inhibitor group as seen in the previous study.[52] It is important to stress that longer follow-up is needed to determine the rate of decline in GFR with confidence.

Our findings of a larger reduction in albuminuria with an ACE inhibitor compared to a calcium antagonist is thus in accordance with most studies, especially of short-acting dihydropyridines.[122,123] Demaire and Bakris[138] also reported that dihydropyridines increased proteinuria in Type 2 DM patients with proteinuria whereas a non-dihydropyridine (diltiazem) reduced it. The long-term effect on diabetic nephropathy in Type 1 DM is at present unknown, as the effect on rate of decline in GFR, or any other hard end point, has not been reported. In Type 2 DM patients with diabetic nephropathy Bakris *et al.*[139] have performed the only long-term study comparing an ACE inhibitor (n = 18) with a calcium antagonist (non-dihydropyridine type) (n = 18) and sympatholytic drugs (n = 16) in patients followed for 63 (8) months. They demonstrated similar rates of decline in creatinine clearance in the ACE inhibitor and calcium antagonist treated patients (1.0 and 1.4 ml min^{-1} yr^{-1}) whereas the patients in the sympatholytic group had a decline of 3.3 ml min^{-1} yr^{-1} ($p < 0.05$). In non-diabetic renal disease a 3-year study of 142 patients found similar rate of decline in GFR when the calcium antagonist nifedipine and the ACE inhibitor captopril were compared.[140]

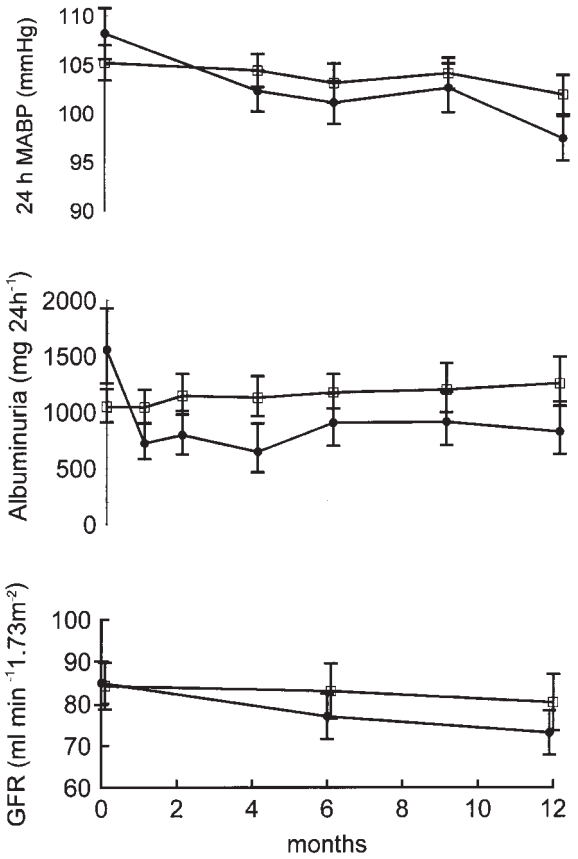

Figure 5. Mean course of mean arterial blood pressure, albuminuria, and GFR during long-term ACE inhibitor treatment with an ACE inhibitor (•, $n = 25$) or a calcium antagonist (□, $n = 24$) in hypertensive Type 1 DM patients with diabetic nephropathy. Bars represent SEM (×/÷ antilog SE for albuminuria) (adapted from Rossing *et al.*[137])

Animal studies have also suggested that ACE inhibitors may be superior to conventional antihypertensive agents due to a reduction in glomerular capillary hydraulic pressure[55] in reducing albuminuria, and also due to effects independent of the intraglomerular pressure, perhaps an anti-proliferative effect,[93] or an effect on glomerular capillary barrier size and charge selectivity.[95,96] Calcium antagonists of the dihydropyridine type may increase the intraglomerular capillary pressure due to a preferential dilation of the afferent glomerular arteriole,[138] whereas non-dihydropyridines have been reported to lower intraglomerular pressure.[141] It has been suggested that the new long acting dihydropyridine calcium antagonists are superior to the short acting such as capsular nifedipine, due to less oscillations in pressure and less activation of the sympathetic nervous system.[142] The beneficial effect of calcium antagonists has been suggested to be mediated by a reduction in kidney and glomerular growth.[143,144] It is therefore possible that the antiproteinuric effect is not a good predictor of the effect of calcium antagonists; on the other hand it is also possible that GFR initially is preserved by an increase in intraglomerular pressure while the structural deterioration continues or even accelerates. It has been suggested that a combination of ACE inhibitors and calcium antagonists is superior to either agent alone.[145,146] The ACE inhibitors and calcium antagonists have the advantage of being metabolically neutral, in contrast to the β-blockers, which also have the disadvantage of interfering with hypoglycemia recognition and recovery. There are few side effects with the ACE inhibitors, but dry cough is a problem in 5–10 %. This may be avoided with the angiotensin receptor antagonists, which also have the advantage of blocking Angiotensin II formed by alternative pathways,[147] but does not inhibit the degradation of kinins. The long-term efficacy of the angiotensin II receptor blockers has not yet been tested in diabetic nephropathy. A diuretic agent almost always has to be added to any other antihypertensive agent due to the sodium and water retention characterizing hypertension in diabetic nephropathy.[44,45]

In summary, it is of major importance to treat even mild hypertension in diabetic nephropathy. It is of secondary importance which agent is used, but ACE inhibitors seems superior to conventional treatment especially in advanced nephropathy. Furthermore, ACE inhibitors have fewer side effects. The long-term effect of the various groups of calcium antagonists, and of new agents such as angiotensin II receptor antagonists, needs to be clarified. It also needs to be addressed when to start treatment and the therapeutic goal needs to be determined.

Lipid Lowering Treatment

Patients with diabetic nephropathy have an increased risk for cardiovascular disease. Thus intervention against hyperlipidaemia is probably warranted in order to reduce mortality and morbidity. Dietary advice and reduction of nephrotic range proteinuria with ACE inhibitors and correction of severe hyperglycaemia should be the initial intervention.

However, most studies designed to evaluate the benefit of pharmacological intervention against hyperlipidaemia have excluded diabetic patients. The recent Scandinavian Simvastitin Survival Study evaluating the effect of simvastitin in patients with angina pectoris or myocardial infarction and elevated serum cholesterol demonstrated a significant reduction in major coronary events in 202 diabetic patients.[148]

Animal models and studies in humans have also suggested that elevated serum cholesterol is a promoter of progression in diabetic nephropathy (see above). We evaluated whether a reduction in hypercholesterolaemia with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HMG-CoA reductase inhibitor) simvastitin diminished albuminuria in Type 1 DM patients with diabetic nephropathy. We performed a randomized, double-blind, placebo-controlled study using simvastitin for 12 weeks in nephropathic patients with serum cholesterol $>5.5\ \text{mmol}^{-1}$. We observed a significant reduction in serum total cholesterol from 6.4 (SD 0.9) to 4.8 (0.7) mmol^{-1} in the simvastitin group whereas there

was no change in the placebo group. Similarly LDL-cholesterol and apolipoprotein B were significantly reduced in the intervention group compared to placebo. There was no effect of simvastitin on albuminuria or GFR, and other confounders such as arterial blood pressure and hemoglobin A_{1c}, and antihypertensive treatment was unchanged. There was a non-significant reduction in albuminuria in both groups, stressing the importance of a control group.

Lowering of plasma lipids with lovastatin or clofibric acid reduces intraglomerular pressure, albuminuria, and development of glomerulosclerosis in animal models.[149,150] In addition to the beneficial effect on intraglomerular pressure, this could be due to reduced mesangial deposition of lipoproteins, or due to inhibited production of mevalonate, a product of HMG-CoA reductase stimulating cell proliferation.[151] Small uncontrolled studies have suggested a beneficial effect on proteinuria in non-diabetic[152] and diabetic (Type 2 DM) renal disease.[153] The beneficial effect was apparent after a few weeks, suggesting that the lack of effect in our study was not due to too short a treatment period. A randomized double blind study of 36 weeks treatment with simvastitin in microalbuminuric Type 2 DM patients found no effect on albuminuria or GFR.[154] A single blind randomized study for 2 years in Type 2 DM patients with micro- and macroalbuminuria (16 on lovastatin and 18 on placebo) postulated a beneficial effect on nephropathy progression.[155] Although GFR declined compared to baseline in the placebo group and not in the lovastatin group, GFR, albuminuria, and serum creatinine were not different between the two groups at any point in time and the differences from baseline to treatment period in each group were not compared. Thomas *et al.*,[156] in a 24-week randomized double-blind placebo-controlled study in patients with significant proteinuria due to non-diabetic renal disease, observed no effect on proteinuria or GFR. A properly conducted long-term study evaluating the effect on decline in GFR or perhaps morphology is needed.

Dietary Intervention

Dyerberg and Bang,[157] originally observed that Greenland Eskimos with a traditionally high consumption of seal and seafood have a low prevalence of atherosclerosis and a low mortality from myocardial infarction. Epidemiological and interventional studies in the general population have shown an inverse dose response relation between fish consumption and death from coronary heart disease.[158,159] Studies in non-diabetic renal disease have suggested that dietary intervention with n-3 polyunsaturated fatty acids (fish oil) reduces albuminuria[160,161] and arterial blood pressure[160,162] preserves kidney function[161,162] and improves dyslipidaemia.[160,163] We tested whether it would also be efficacious in the treatment of normotensive Type 1 DM patients with diabetic nephropathy in a 1-year double-blind randomized study comparing cod-liver oil with olive oil.[85] Normotensive patients were selected because no treatment modality has been efficacious in this group, and because antihypertensive treatment could bias the outcome of the study. Apart from a reduction in serum triglyceride and an increase in the platelet content of n-3 fatty acids (our measure of compliance), there were no differences between the groups in changes in albuminuria, GFR, 24 h ambulatory blood pressure, HbA_{1c}, and other lipids and lipoproteins.[85] In addition there was no effect on transcapillary escape rate of albumin or on the coagulation system (B. Myrup, personal communication).

Previous short-term studies of fish oil in a small number of normo- micro- or macroalbuminuric Type 1 DM diabetic patients have yielded conflicting data on urinary albumin excretion.[164–167] The discrepancies could relate to differences in the fish oil preparations or the type of patients. It is also possible that some of the effects observed in short-term studies are transient, as a study from our clinic[164] using almost the same oil as in our study for 8 weeks in a cross-over study in Type 1 DM patients with micro- and macroalbuminuria did find a reduction in blood pressure and transcapillary escape rate of albumin and an improvement in the lipid profile[164] in contrast to our long-term study. Another possibility is that antihypertensive treatment influenced the outcome in the latter study or that the lack of response in our study was due to absence of hypertension. A recent metanalysis of the effect of fish oil on arterial blood pressure in diabetic and non-diabetic patients reported that a reduction in arterial blood pressure was only observed in hypertensive patients.[168] Finally the failure to demonstrate beneficial effects in our study could be due to the small number of patients in combination with the large variation in albuminuria. However, the trend, if any, was contrary to being beneficial. The present study does not allow for any conclusions regarding the long-term effect on the cardiovascular risk.

In conclusion, our 1-year study of fish oil provided no evidence of a renoprotective effect. Although a study of longer duration is needed to definitely exclude such an effect, none of the potential progression promoters or predictors were modified.

Improved Metabolic Control

A salutary effect of strict glycaemic control on the prevention of diabetic nephropathy has been demonstrated in small randomized trials summarized by Wang *et al.*[25] and in the Diabetes Control and Complications Trial (DCCT),[22] although the DCCT study could not demonstrate an effect in patients with microalbuminuria at study onset ($>28\ \mu g\ min^{-1}$).[169] A British study of microalbuminuric patients also failed to demonstrate an effect of intensified glycaemic control in microalbuminuric patients.[170] In contrast, the effect of near normalization of blood glucose on the progression of diabetic nephropathy is unknown. The only available study comprises 6 patients[79] and strict glycaemic control did not improve

rate of decline in GFR. We,[52] and others,[57,78] have demonstrated an association between low HbA_{1c} and a slow deterioration in kidney function, but this association does not prove causality. It has been argued that after initial damage to the kidney, removal of the initial noxious stress would not affect the further course of deterioration in nephropathy, a point of no return. We need long-term randomized studies evaluating the effect of strict glycaemic control.

Dietary Protein Restriction

Dietary restriction in protein delays or prevents the progression of diabetic glomerulopathy in experimental animals, reducing intraglomerular pressure through an increase in afferent glomerular capillary resistance.[171,172] This effect is probably mediated through different prostaglandins affecting renal haemodynamic, but also an effect mediated through growth factors (PDGF-A and -B, and TGF-β) affecting glomerulosclerosis has been suggested. Two long-term studies in Type 1 DM patients with diabetic nephropathy have suggested that dietary protein restriction reduces the rate of decline in GFR and the albuminuria.[73,173] However, the conclusions have been questioned.[174,175] Walker *et al.*[73] studied the rate of decline in GFR before and during dietary protein restriction, but antihypertensive treatment was initiated or intensified in 9 of 19 patients during protein restriction. In the study by Zeller *et al.*,[173] a parallel study of 35 patients, the decline in GFR in the group receiving normal diet was remarkably high (12 ml min^{-1} yr^{-1}), comparable to the decline in patients without antihypertensive medication, whereas the progression in the group on low protein diet was comparable to patients on normal diet but receiving antihypertensive treatment (3.1 ml min^{-1} yr^{-1}).

The largest study of the effect of dietary protein restriction on the progression of chronic renal disease excluded patients with Type 1 DM and included only 3 % (~25 patients) with Type 2 DM.[176] This randomized controlled trial was unable to demonstrate an effect of protein restriction on the rate of decline in GFR after 3 years; however, in the protein restricted group an initial fast decline in GFR was followed by a slower decline in GFR. Thus it was suggested that if the study had been prolonged the low protein diet would have been beneficial.

In conclusion, the effect of restriction of dietary protein in diabetic nephropathy needs to be determined in a long-term randomized trial. Furthermore it needs to be determined if the source of protein matters (animal/vegetable).

Prognosis in Type 1 DM with Diabetic Nephropathy

The relative mortality in Type 1 DM patients, compared with the background population, is increased 40 times in Type 1 DM patients with proteinuria vs ~2 times in Type 1 DM patients without frank proteinuria.[177] The spontaneous course of diabetic nephropathy in patients solely treated with insulin (i.e. without treatment of hypertension and end-stage renal disease) has a median survival of 5–7 years after onset of persistent proteinuria.[1,178] The primary cause of death is end-stage renal failure (66 % of the patients).[1] However persistent proteinuria is not only a marker of renal involvement, but also an indicator of generalized microangiopathy,[179] and macroangiopathy.[180] Persistent proteinuria is also a risk marker for increased cardiovascular mortality.[10] The use of early aggressive antihypertensive treatment in Type 1 DM patients with diabetic nephropathy has had a major impact on their survival.[181–183] Historical control groups, where antihypertensive medication was rare, had a mortality of 50–77 % 10 years after the onset of persistent proteinuria.[1,2,178] The 50 %[2] is a minimum estimate only including renal death. This should be compared to a 10-year mortality of only 18 %[181] and a median survival of more than 16 years[183] in patients in whom antihypertensive treatment had been started at a diastolic blood pressure exceeding 95 mmHg. Mathiesen *et al.*[182] demonstrated a reduction in 8-year mortality from 52 % to 13 % with the introduction of antihypertensive treatment. As these studies in small incidence cohorts used historical control groups, the effect of other factors cannot be totally excluded, but the improvement in median survival time corresponds well with the improvement in mean rate of decline in glomerular filtration rate caused by antihypertensive treatment. Improvement in glycaemic control is an unlikely explanation as the HbA_{1c} was >9 % during antihypertensive treatment.[181,182]

We performed a 10-year prospective follow-up study of a cohort of Type 1 DM patients from Hvidöre Hospital identified in 1984.[20] The aim was to identify predictors of mortality in Type 1 DM patients. We found that increased albuminuria was associated with increased all cause and cardiovascular mortality as previously demonstrated,[10,177,184] and that microalbuminuria *per se* only increased the mortality slightly.[185] A 10-fold increase in albuminuria increased the risk for all cause mortality with 45 %, and the relative risk for cardiovascular death was 2.97 in the presence of persistent proteinuria compared to normoalbuminuria, when correction for other putative risk factors were made. This is comparable to the findings of Stephenson *et al.*,[184] but lower than the findings of Borch-Johnsen and Kreiner.[10] However the patients studied by Borch-Johnsen were younger at onset of diabetes, calender year of diagnosis was earlier when compared to our study, antihypertensive treatment was used less frequently, and the relative risk was not adjusted for hypertension and smoking.

Has the introduction of antihypertensive treatment changed the survival in a large, unselected cohort of patients with diabetic nephropathy? We followed 263 patients with diabetic nephropathy for up to 20 years.[185] From the Hvidöre 1984 cohort,[20] the onset of diabetic

nephropathy in 165 patients with nephropathy in 1984 was determined retrospectively. During follow-up, an additional 98 patients, initially normo- or microalbuminuric, developed nephropathy. We demonstrated a median survival of 14 years (95 % confidence interval: 12–17) after onset of diabetic nephropathy[185] (see Figure 6), in good agreement with the smaller studies.[181–183] End-stage renal disease was the cause of death in 35 % of the patients with nephropathy in 1984, cardiovascular disease in 33 %; 32 % died for other reasons. This change in the pattern of causes of death with the postponement of end-stage renal disease by a reduction in the rate of decline in kidney function increases the importance of identification and treatment of the multiple cardiovascular risk factors often present in patients with diabetic nephropathy such as dyslipidaemia, smoking, and abnormal procoagulant state.

The future challenge will be to focus on identification and treatment of cardiovascular diseases in diabetic nephropathy in order to improve the prognosis further. The effect on survival of other putative treatment modalities which could diminish the loss of kidney function will also have to be addressed, and the survival in our cohort of patients with nephropathy, where antihypertensive treatment has been the major intervention apart from insulin, and where blood pressure and glycaemic control are known, can serve as a reference group if parallel studies can not be performed.

In the initial studies reporting a beneficial effect of antihypertensive treatment on survival the treatment was primarily conventional antihypertensive medication (diuretics, β-blockers and hydralazine), which was also the case in our clinic-based study.[185] In a prospective study on the effect of antihypertensive treatment with the ACE inhibitor captopril (often in combination with diuretics) in 18 hypertensive Type 1 DM patients we evaluated the survival from onset of diabetic nephropathy.[52] We observed a 10 years' mortality of 11 %, comparable to the mortality on conventional treatment (see Figure 7). Due to the small number of patients it was not possible to evaluate whether the survival was significantly different from the survival on conventional treatment. Recently the Collaborative Study Group of Angiotensin Converting Enzyme Inhibition with Captopril in Diabetic Nephropathy[118] demonstrated a risk reduction for occurrence of death or progression to dialysis or transplantation of 56 % (95 % CI: 26 to 80 %, $p = 0.002$) in the 102 patients with serum creatinine levels greater than 133 μmol l^{-1} and a risk reduction of 46 % (95 % CI: −22 to 76 %, $p = 0.14$) in 307 patients with serum creatinine <133 μmol l^{-1}, in Type 1 DM patients treated with captopril versus placebo. All hypertensive patients ($n = 308$) in this trial received conventional antihypertensive treatment plus captopril or placebo therapy. In a non-randomized but parallel 5-year study Sawicki *et al.*[186] demonstrated that intensified antihypertensive treatment using cardioselective β-blockers, calcium antagonists, and diuretics (goal: arterial blood pressure below 140/90) improved survival compared to the usual treatment for hypertension (mainly diuretics, ACE inhibitors, and calcium antagonists).

The costs of caring for the Type 1 DM patients reaching end-stage renal disease are high and rising (US$ 1.8 billion per year in the US) and the prognosis is poor. Renal transplantation is the preferable treatment but the supply of kidneys is insufficient. However it has been increasingly common to use kidneys from living unrelated donors or spouses, and living-donor kidneys actually

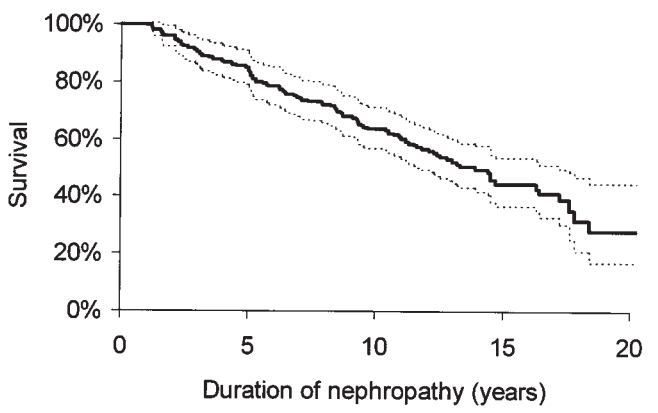

Figure 6. Survivor function from onset of overt diabetic nephropathy with simple confidence intervals based on the Nelson-Aalen hazard and left truncated data (patients with overt nephropathy at baseline are left truncated and contributes from the time corresponding to the duration of overt nephropathy at January 1, 1985). Two hundred and sixty-three patients with Type 1 DM and diabetic nephropathy, 94 deaths (adapted from Rossing *et al.*[185])

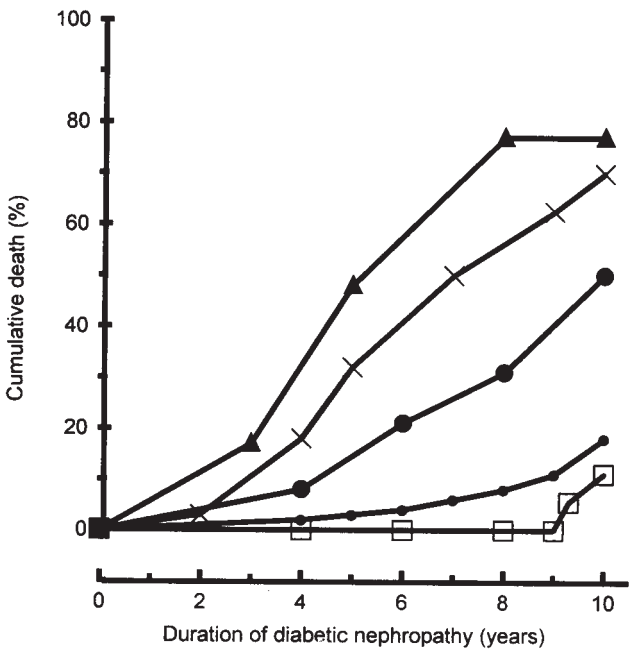

Figure 7. Deaths from diabetic nephropathy (natural history) in Type 1 DM patients (▲, $n = 45$, Knowles;[178] ×, $n = 360$ Andersen *et al.*;[1] ●, $n = 67$, Krolewski *et al.*[2]) compared with those who had effective antihypertensive treatment without ACE inhibition (•, $n = 45$, Parving *et al.*[181]) and with ACE inhibition (□, $n = 18$, Parving *et al.*[52])

have a higher survival compared to cadaveric kidneys despite a higher degree of HLA mismatch.[187] A recent study suggested that transplantation of a kidney alone was superior to a combined kidney and pancreas transplantation with a 3-year survival of 90 % vs 68 %, respectively.[188] Compared to this the survival at 24 months is approximately 60 % on continuous ambulatory peritoneal dialysis and 40 % on haemodialysis.[189]

In conclusion, the presence of diabetic nephropathy is associated with an increased all cause and cardiovascular mortality. The early and aggressive use of antihypertensive treatment has increased the median survival time from 5–7 to 14–17 years. This has been followed by a change in the pattern of death causes. Reducing the mortality due to end-stage renal failure makes it mandatory to focus on identification and treatment of cardiovascular risk factors and diseases.

- Aggressive antihypertensive treatment has improved the survival in diabetic nephropathy.
- Cardiovascular disease becomes increasingly important as uraemia is avoided/postponed.

Summary and Future Perspectives

Diabetic nephropathy is the most serious long-term complication in Type 1 DM associated with generalized micro- and macrovascular damage, and increased all cause and cardiovascular mortality. We have not been able to confirm that the incidence of diabetic nephropathy is declining. Future research will have to evaluate whether the experience from promising trials using strict glycaemic control and ACE inhibitors in the prevention of diabetic nephropathy can be implemented in the daily clinic.

Once a patient has developed diabetic nephropathy, it is important to monitor the changes in renal function by regular reliable determinations of GFR, once persistent albuminuria >300 mg 24 h^{-1} has been observed.

Early and aggressive antihypertensive treatment is the most important therapy, apart from insulin, in patients with diabetic nephropathy, at least when hypertension is present. Although the average rate of decline in GFR is markedly reduced, the efficacy varies among patients, as does the underlying rate of decline. Change in albuminuria shortly after onset of antihypertensive treatment is a good predictor of the long-term rate of decline in GFR but whether changes in albuminuria can predict the efficacy of all antihypertensive treatments and other treatment modalities needs to be determined. In the future the genetic susceptibility to diabetic nephropathy should be evaluated further.

Antihypertensive treatment in hypertensive Type 1 DM patients with diabetic nephropathy is efficacious in slowing down the deterioration in kidney function. This effect is maintained in long-term studies. Survival has improved with the use of antihypertensive agents. A comparison of the efficacy of ACE inhibition and calcium antagonism is under study. Current data on the effects of the HMG CoA reductase inhibitor simvastitin, and of dietary modification with fish oil supplementation, have not demonstrated useful effects on renal function and albuminuria. Nevertheless dyslipidaemia calls for intervention due to the increased cardiovascular risk. Future studies should evaluate the efficacy of long-term lipid lowering drugs, protein restricted diet and of strict glycaemic control, as well as new antihypertensive agents on progression of diabetic nephropathy. The possibilities for intervention using agents inhibiting glycosylation (such as aminoguanidin) or limiting growth factors responsible for mesangial proliferation (decorin that inhibits the proliferative effects of TGF-β1) or PKC inhibitors need to be explored, especially prior to the development, or in the early stages, of nephropathy.

The improved prognosis in diabetic renal disease with an increase in median survival after onset of diabetic nephropathy from 5–7 to 14–17 years augments the importance of cardiovascular morbidity and mortality, now becoming a more predominant complication. The identification and treatment of cardiovascular risk factors thus becomes increasingly critical. Although the prognosis has improved remarkably, the primary goal should be prevention of diabetic nephropathy, as it is unlikely that the increased risks associated with this complication can be totally eliminated.

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